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E-Article

The Balanced Lethal System of Crested Newts: A Ghost of Sex Chromosomes Past?

Christine Grossen,[1,2,*] Samuel Neuenschwander,[1,3] and Nicolas Perrin[1]

1. Department of Ecology and Evolution, University of Lausanne, CH 1015 Lausanne, Switzerland; 2. Institute of Evolutionary Biology and Environmental Studies, University of Zürich, CH 8057 Zürich, Switzerland; 3. Vital-IT, Swiss Institute of Bioinformatics, University of Lausanne, CH 1015 Lausanne, Switzerland

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abstract: Balanced lethal systems are more than biological curiosities: as theory predicts, they should quickly be eliminated through the joint forces of recombination and selection. That such systems might become fixed in natural populations poses a challenge to evolutionary theory. Here we address the case of a balanced lethal system fixed in crested newts and related species, which makes 50% of offspring die early in development. All adults are heteromorphic for chromosome pair 1. The two homologues (1A and 1B) have different recessive deleterious alleles fixed on a nonrecombining segment, so that heterozygotes are viable, while homozygotes are lethal. Given such a strong segregation load, how could autosomes stop recombining? We propose a role for a sex-chromosome turnover from pair 1 (putative ancestral sex chromosome) to pair 4 (currently active sex chromosome). Accordingly, 1A and 1B represent two variants (Y_A and Y_B) of the Y chromosome from an ancestral male-heterogametic system. We formalize a scenario in which turnovers are driven by sex ratio selection stemming from gene-environment interactions on sex determination. Individual-based simulations show that a balanced lethal system can be fixed with significant likelihood, provided the masculinizing allele on chromosome 4 appears after the elimination of the feminizing allele on chromosome 1. Our study illustrates how strikingly maladaptive traits might evolve through natural selection.

Keywords: balanced lethal system, maladaptive trait, sex chromosome turnovers, sex ratio selection.

Introduction

When a female crested newt lays a clutch, nothing will save her from losing half of her investment. Fifty percent of all embryos will stop growing early in development and die within a few days (Rusconi 1821; in Wallace 1987). This developmental arrest syndrome was later recognized to result from a balanced lethal system. Callan and Lloyd (1960) first noticed that chromosome pair 1 in *Triturus cristatus* adults was heteromorphic and that the two variants (1A and 1B) harbored heterochromatic segments that did not form chiasmata in the oocyte lampbrush bivalent stage. Further investigations showed that all nonviable offspring were homozygotes for one of the two variants (i.e., 1A/1A or 1B/1B). The same pattern was found to occur in the related *Triturus marmoratus* but not in the more distant *Triturus alpestris* (Macgregor and Horner 1980). *Triturus marmoratus* and *T. cristatus* shared a common ancestor some 20 million years ago (Arntzen et al. 2007; Steinfartz et al. 2007). Experimental hybridization shows that chromosome 1A from one species and 1B from the other complement each other for larval viability (Sims et al. 1984). Hence, this balanced lethal system is likely to have been inherited from their common ancestor.

How could such a maladaptive trait have evolved and be maintained in the face of natural selection, which is expected to maximize individual fitness? Two main hypotheses have been proposed so far. The first one (Sims et al. 1984; reformulated by Sessions et al. 1988) postulates a "cytogenetic accident" (specifically, unequal genic exchange between the two homologues of an autosomal pair) that occurred in a common ancestor, making crossing over impossible in the region concerned. The several inversions and repeat sequences observed today (e.g., Sims et al. 1984) accumulated on the differential segment of chromosomes 1A and 1B following this arrest of recombination. The question arises, however, how a mutation leading to such an extreme fitness reduction might become fixed in a population. As the model goes, the two haplotypes (1A and 1B) resulting from unequal exchange would each carry at least one duplication and one deletion of genes essential for embryonic development (Sessions et al. 1988). Making the conservative assumption that selection removes only individuals that are homozygous for a deletion, the fitness of haplotypes 1A and 1B (relative to the normal wild-type

* Corresponding author; e-mail: christine.grossen@uzh.ch.

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haplotype 1) is expected to decrease linearly with their respective frequencies p and q (being $1 - p$ for 1A and $1 - q$ for 1B). Such strong negative frequency dependence should quickly eliminate mutant haplotypes, whatever the local effective population size.

The second hypothesis, inspired by the striking similarities with features that normally characterize the nonrecombining segments of sex chromosomes (including inversions, rearrangements, deletions, and accumulation of repetitive sequences), links the origin of the balanced lethal system to sex determination (Wallace 1984, 1987; Wallace et al. 1997; Wallace and Wallace 2000). Chromosome 1 was rapidly discarded as a candidate for sex determination because heteromorphism was found to occur in both sexes (Morgan 1978; Macgregor and Horner 1980). The chromosome pair 4 was then identified as the sex chromosome pair, bearing a male-heterogametic (*XX*/*XY*) system (Sims et al. 1984). Wallace (1987) suggested that 1A and 1B actually represent the two chromosomes of an ancestral *AA*/*AB* sex-determination system. Accordingly, *BB* homozygotes are lethal because B accumulated deleterious mutations along its evolution in the heterogametic sex. This old system was then supplanted by the new *XY* system on chromosome 4, which operated effectively only in the former heterogametic sex *AB*. It is not clear, however, why the *XY* system should only operate in an *AB* context, and how *AA* should become lethal, given that the *A* chromosome normally recombined in the former homogametic sex.

In this article, we formalize an alternative hypothesis, which also relates this balanced lethal system to ancestral sex chromosomes. Specifically, we propose that the two homologues 1A and 1B represent two forms (Y_A and Y_B, respectively) of the nonrecombining sex chromosome from an ancient *XX*/*XY* system. We will first outline the main steps in the argument and then formalize the argument in a simulation model.

Nonrecombining Y chromosomes necessarily accumulate deleterious recessive mutations because of enhanced genetic drift, selective sweeps, background selection, and Muller's ratchet (Charlesworth and Charlesworth 2000; Bachtrog 2006; Ellegren 2011). Several Y haplotypes (i.e., fixed for different mutations) may segregate within populations. Such a situation has been well documented, for instance, in the guppy *Poecilia reticulata*, where at least three different Y variants have been shown to coexist in natural populations (Haskins et al. 1970 and references therein). These haplotypes code for different male coloration morphs and are thus possibly maintained by frequency-dependent selection occurring through female mate choice. When experimentally mating sex-reversed *XY* females with *XY* males from different haplotypes, 25% *YY* offspring are produced, which develop into fully viable and fertile males when heterozygous for the Y haplotypes but are lethal when homozygous (Haskins et al. 1970 and references therein). Similar processes occur in female-heterogametic (*ZW*/*ZZ*) populations of *Rana rugosa*: different populations have fixed different W haplotypes, so that *WW* individuals are viable when their two W chromosomes stem from different populations but not when they are from the same population (Miura et al. 2012). This necessarily implies that each haplotype has fixed one or more recessive lethal mutations (e.g., loss of function of some housekeeping genes) and that different mutations occur in different haplotypes.

Sex reversal is easily triggered by temperature in many cold-blooded vertebrates, presumably because of thermal dependence in the expression of genes (or activity of enzymes) involved in the sex-determination cascade (see, e.g., Grossen et al. 2011). This is in particular true of crested newts, in which high temperatures have a masculinizing effect, while low temperatures have a feminizing effect (Wallace et al. 1999; Wallace and Wallace 2000).

Let us assume that an ancestral newt population, harboring Y haplotypes diverging in terms of inversions and deleterious mutations (Y_A and Y_B), experienced a feminizing temperature shift (due, e.g., to climatic changes or range expansion), so that increasingly large numbers of XY_A or XY_B genotypes developed into females. When mating with normal XY_A or XY_B males, these females generated (among other offspring) lethal Y_AY_A and Y_BY_B homozygotes, as well as viable Y_AY_B heterozygotes. The balanced lethal system now fixed in *T. cristatus* was thereby produced. For the same reason (sex reversal), this temperature shift also generated biased sex ratios (namely, an excess of females), thereby inducing a selective pressure for any masculinizing mutation able to restore even sex ratios (Grossen et al. 2011). As we will formalize below through individual-based simulations, this new mutation could spread to establish the new male-heterogametic system nowadays found on chromosome 4 in crested newt lineages, while still maintaining the Y_AY_B balanced lethal system trapped on the ancestral chromosomal pair 1.

Methods

Conceptual Model

Sex-determination mechanisms can be modeled, in a quantitative genetics framework, as a continuum between purely genetic processes on the one hand and purely environmental processes (e.g., temperature; TSD) on the other hand (Sarre et al. 2004; Grossen et al. 2011). Specifically, sex qualifies as a threshold trait, underlain by a liability factor (e.g., a sex hormone). Any individual will develop into a male if its liability trait value A exceeds the

threshold (z) and into a female otherwise (fig. 1). This liability trait value $A_{IJ,T}$ depends on the individual genotype IJ, on the mean local temperature T, and on individual deviation from this mean, stemming from microenvironment differences during the sensitive period of embryonic development. Hence, the phenotypic variance in the liability trait within populations has a genetic component (stemming from the coexistence of different genotypes) and an environmental component, assumed to be normally distributed with mean 0 and standard deviation σ_E.

Genotypes can be defined by reaction norms (assumed to be linear in fig. 1), representing the amount of the liability trait produced by this genotype as a function of temperature. Hence, depending on local temperature, a given genotype may develop as either male or female. Temperature shifts (e.g., due to climatic changes or range expansion) will thus generate biases in sex ratios. As a consequence, sex ratio selection will induce a change in frequency of sex-determination alleles. From figure 1, for instance, in the absence of M, the masculinizing Y allele is expected to rise in frequency from 0.25 to 0.5 with a two-unit drop of temperature from its initial value. Further drops will ultimately select for a new sex-determination system (for details, see Grossen et al. 2011; for similar conceptualizations, see, e.g., Bulmer and Bull 1982; Quinn et al. 2007, 2011; Pen et al. 2010).

Implementation

We assumed linear and parallel norms of reaction, modeled as $\alpha_{IJ,T} = \beta(T - \tilde{T}_{IJ})$, where $\alpha_{IJ,T} = (A_{IJ,T} - z)/\sigma_E$ is the standardized liability trait value for genotype IJ, β is the standardized slope (change in standardized liability trait per unit change in temperature, fixed to 1 without loss of generality), and $\tilde{T}_{IJ}$ is the pivotal temperature for genotype IJ (i.e., the temperature at which this genotype produces males and females in equal proportions).

Sex genotypes were defined at two unlinked loci. The initial sex-determining locus (on chromosome 1) had one feminizing allele X and two masculinizing alleles Y_A and Y_B. The threshold z was arbitrarily set to 0, and allelic values at initial temperature conditions ($T = 0°$) were fixed to -1 for X and $+3$ for both Y_A and Y_B. Effects were additive, so that XX (genotypic value -2; yellow in fig. 2) developed into females, while XY_A and XY_B (genotypic values $+2$; pale green in fig. 2) developed into males. The second locus (on chromosome 4; horizontal axis in fig. 2)

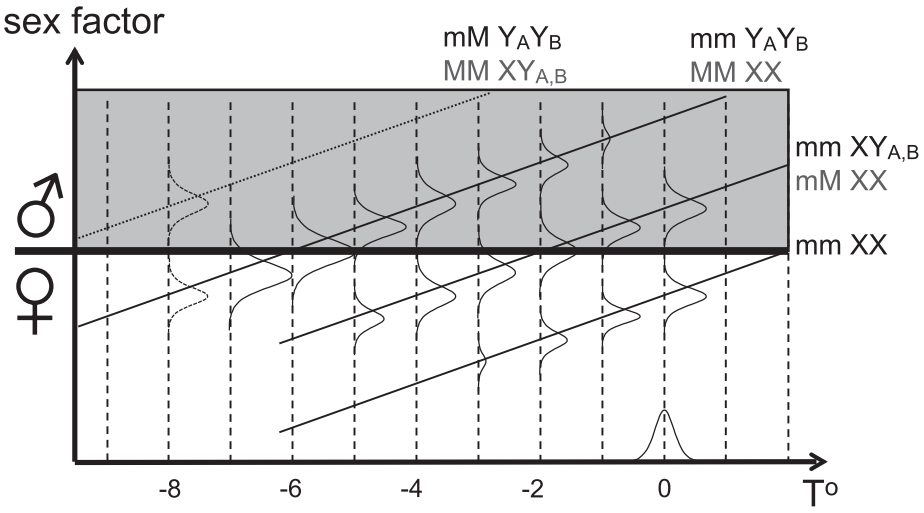


Figure 1: Quantitative genetics model of sex determination with gene-environment interactions. The liability trait (sex factor) produced by genotype IJ increases with temperature $T°$ (norms of reaction are assumed linear and parallel with slope $\beta = 1$). Individual differences within populations (microenvironment differences during the sensitive period of embryonic development; Gaussian curve on the horizontal axis) translate into individual deviation from the genotypic mean (Gaussian curves on the vertical axis). Individuals develop into males if the sex factor exceeds a threshold (bold horizontal line) and into females otherwise. At initial conditions ($T = 0°$), genotypic values define a male-heterogametic system with $mmXX$ females and $mmXY_{A,B}$ males. Temperature decreases will lead to sex ratio selection, favoring a masculinizing mutation (M). If M appears before the loss of X, it goes to fixation and the initial XX/XY system is restored (genotypes in gray). If M appears after X is lost, a new male heterogametic system evolves on chromosome 4, with the fixation of a balanced lethal system on chromosome 1 (mMY_AY_B, mmY_AY_B).

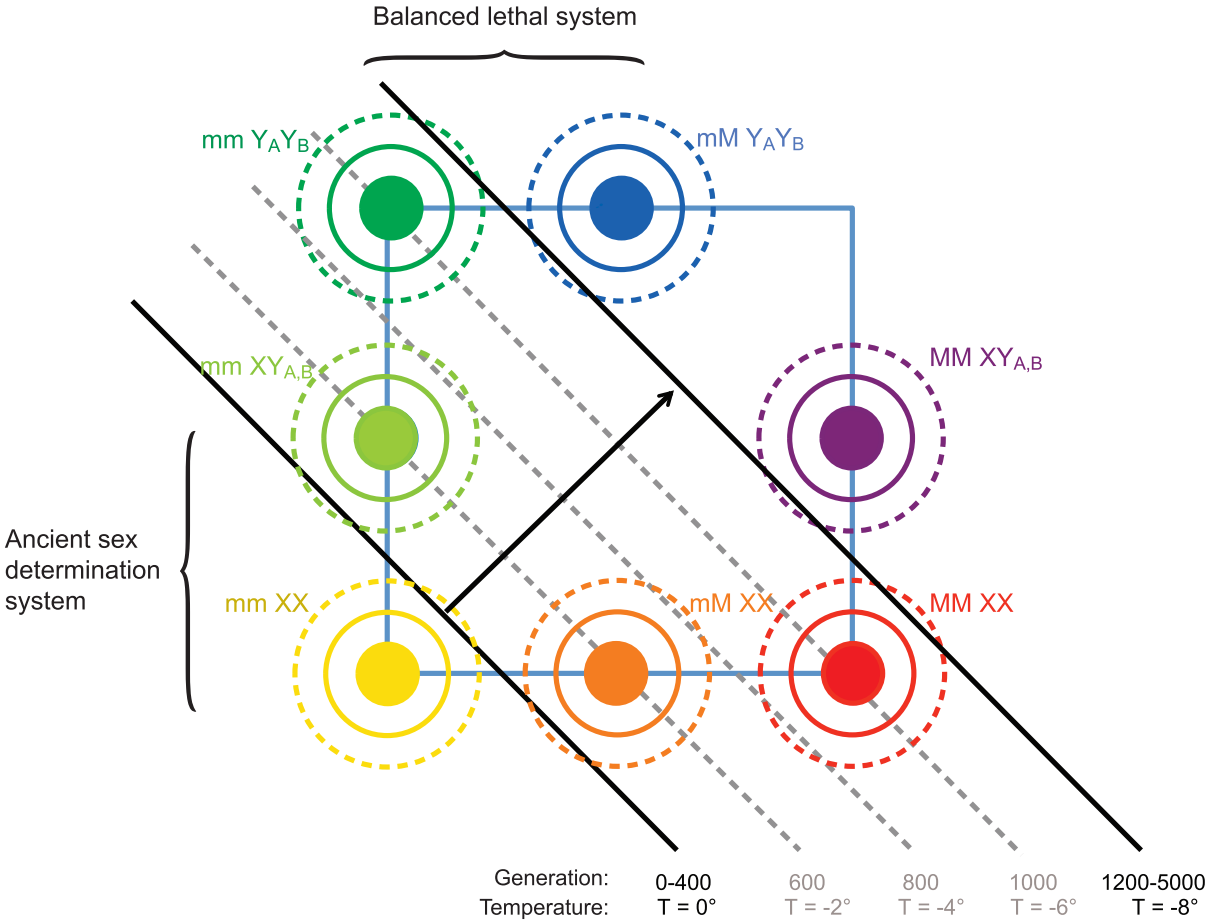


Figure 2: Sex genotypes in a gradient of femaleness (bottom left) to maleness (top right). From the *mmXX* genotype (yellow, bottom left), masculinization may occur by replacing either X by Y (vertical axis) or m by M (horizontal axis). Circles around genotypic values represent the environmental variance in the liability trait (Gaussian distribution of phenotypes around the genotype average). At initial conditions, the threshold is given by the diagonal straight line at $T = 0°$. The initial recurrent pair is male heterogametic *mmXX*/*mmXY* (the yellow–pale green pair). Most *mmXX* individuals (yellow) are below the threshold and thus develop as females (except for the few crossing the threshold line), and most *mmXY* individuals (pale green) are above the threshold and thus develop as males. The change in environmental conditions (decreasing temperatures) is figured by the progressive displacement of the threshold line toward the male corner, from $T = 0°$ to $T = -8°$ (arrow). In scenario 1, mutations to M are not allowed, so that the only possible response to the environmental change is an upward shift (vertical axis), first toward a female-heterogametic *mmXY*/*mmYY* (pale and dark green), then to *mmYY* temperature-dependent sex determination (TSD; dark green), both systems suffering from the Y_AY_B segregation load. At the final temperature ($T = -8°$), populations may still keep the pale green *mmXY* female genotype, lose the X entirely (TSD with fixed *mmYY*), or become extinct, depending on whether population size and environmental variance allows production of a few males. In scenario 2, mutations to M will first generate an alternative male-heterogametic recurrent pair *mmXX*/*mMXX* (the yellow-orange pair). As temperature decreases to $T = -4°$, two female-heterogametic pairs are possible: *mmXY*/*mmYY* on the one hand (pale and dark green, vertical axis), and *mMXX*/*MMXX* on the other hand (orange and red, horizontal axis). However, they are not equivalent: the first one suffers from the segregation load Y_AY_B, so that the latter is more likely to evolve. At $T = -6°$, both *mmYY* (dark green) and *MMXX* (red) produce even sex ratios (hence allowing TSD), but for the reason already mentioned, *mmYY* is unlikely to evolve. At $T = -8°$ (final temperature), the male-heterogametic pair *MMXX*/*MMXY* is favored (red and violet) but can only be reached by populations that did not lose their Y in the process (mostly large ones). Other populations will either be trapped in the red *MMXX* TSD (with female-biased sex ratio) or become extinct if environmental variance is too small to allow production of a few males. In scenario 3, the system will have reached the ending situation of scenario 1 before a mutation to M occurs. In cases where the X allele was maintained (*mmXY*, pale green genotype), the system quickly shifts toward the high-fitness male-heterogametic pair *MMXX*/*MMXY* (red and violet). Otherwise, it is trapped in the balanced lethal, male-heterogametic *mmYY*/*mMYY* system (dark green and blue).

was initially fixed for allele m (allelic value 0) but allowed to mutate to a masculinizing state M (allelic value +4).

We assumed simple life cycles with nonoverlapping generations and constant population sizes. Reproduction occurred by choosing randomly, for each offspring, one father and one mother from the parental generation with replacement (which amounts to a promiscuous mating system) and reiterating this process until reaching the carrying capacity (newts have often been described as lek breeding, and females are known to mate multiply; see, e.g., Rafinski 1981; Verrell and McCabe 1988; Hedlund and Robertson 1989; Halliday 1998; Jones et al. 2002*a*, 2002*b*; Rafinski and Osikowski 2002). Y_AY_A and Y_BY_B were defined as lethal and removed after reproduction.

Simulations

Simulations were run with a modified version of quantiNemo 1.0.3 (Neuenschwander et al. 2008). After a burn-in of 400 generations at starting conditions (arbitrarily fixed to $T = 0°$), temperature was decreased to a final value of $T = -8°$, reached after 1,200 generations, by steps of one temperature unit every 100 generations (standard) or 0.1 every 10 generations (smooth). The smooth temperature change led to the same outcome as the standard change (data not shown).

At initial conditions ($T = 0°$), alleles X, Y_A, and Y_B were segregating on chromosome 1, while m was fixed on chromosome 4. In a first set of simulations, this locus was kept fixed for m (no mutation to M allowed) in order to investigate the evolution of the system under a climatic change in the absence of turnover. In a second set, we allowed masculinizing mutations to occur (at a rate of 10^{-4} or 10^{-5}) right from the beginning. In the third set, this masculinizing mutation was allowed only after the climatic transition had occurred (from generation 3,000, $\mu = 10^{-4}$, corresponding to a very low mutation rate or a combination of several mutations).

For each set of simulations, we tested different carrying capacities ($N = 50$, 100, 500, 1,000, 5,000, and 10,000) and environmental variances (σ_E^2 from 0.3 to 4.2, steps 0.3). We also tested more extreme values of σ_E^2 (from 10^{-7} to 40.96), which produced qualitatively similar results (data not shown).

Results

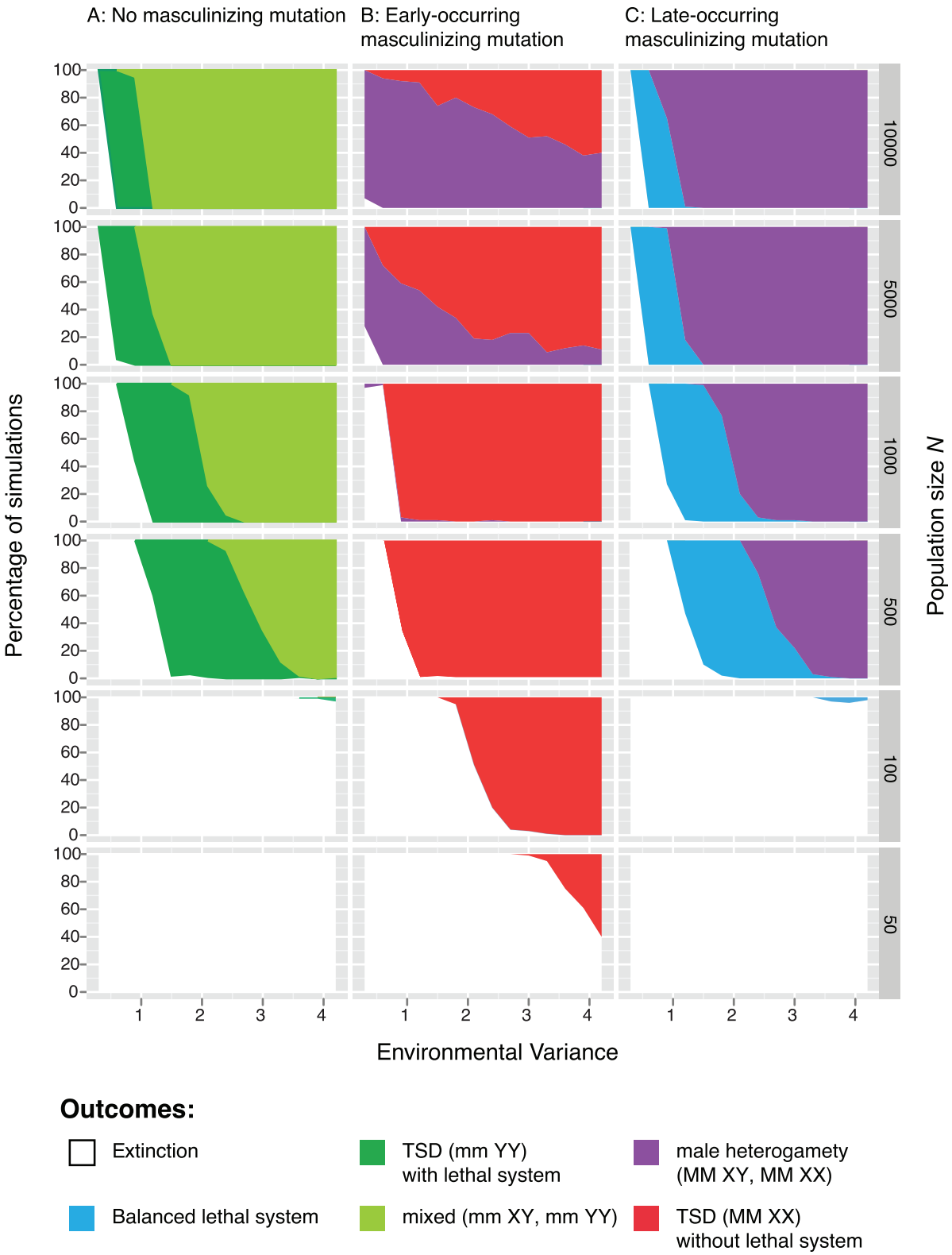
No Masculinizing Mutation

At initial conditions ($T = 0°$), females were *mmXX* (yellow in fig. 2), males $mmXY_A$ or $mmXY_B$ (pale green in fig. 2), sex ratios were equal, and sex reversal was absent (except for a large environmental variance). A first temperature drop ($T = -2°$) generated sex-reversed $mmXY_A$ and $mmXY_B$ females, which produced 25% viable sons (mmY_AY_B) when mating with $mmXY_B$ or $mmXY_A$ males and 25% lethal sons (mmY_AY_A or mmY_BY_B) when mating with $mmXY_A$ or $mmXY_B$ males, respectively. After another drop ($T = -4°$), $mmXY_A$ and $mmXY_B$ genotypes mostly developed into females, while most adult males were mmY_AY_B (dark green in fig. 2). Hence, 25% of offspring died (being either mmY_AY_A or mmY_BY_B depending on whether males mated with a $mmXY_A$ or a $mmXY_B$ female).

With a further temperature drop ($T = -6°$), half of the mmY_AY_B genotypes developed into females, producing 50% lethal offspring when mated with mmY_AY_B males. This system evolved toward pure TSD when X was lost (which often occurred by drift in small populations). However, X had a chance to survive at large σ_E^2 values, because a few *mmXY* then developed as males, with higher fitness than *mmYY* males (which produced more lethal *YY* homozygotes). Finally, following the last temperature drop ($T = -8°$), mmY_AY_B developed preferentially in females. This induced large female biases in sex ratios, leading to extinctions at small N and/or σ_E^2 values (white areas in fig. 3*A*). Such extinctions did not occur when large N and/or σ_E^2 permitted the development of at least a few males to rescue the population. This resulted in pure TSD (with 50% offspring mortality and strongly female-biased sex ratios) in the cases in which X had been lost (dark green areas in fig. 3*A*), and a mixed system when large N and/or σ_E^2 allowed X survival (pale green areas in fig. 3*A*).

Early-Occurring Masculinizing Mutation

When the new masculinizing mutation M appeared early in the simulations (before X had any chance to be lost), it progressively increased in frequency as temperature dropped, ultimately becoming fixed in the population. At $T = -4°$, this mutation first allowed evolution toward an alternative female heterogametic system (*mMXX* females and *MMXX* males; orange and red in fig. 2) with the potential to entirely lose Y_A and/or Y_B. At $T = -6°$, *MMXX* homozygotes produced males and females in equal quantities, allowing pure TSD to evolve, with the concomitant risk of losing Y_A and/or Y_B as well. Hence, X was often fixed by drift in small populations. Lower temperatures ($T = -8°$) then restored selection in favor of Y_A and/or Y_B (because *MMXX* increasingly developed into females) but mostly at small environmental variance: high σ_E^2 values increased the probability that a few *MMXX* develop into males (fitter than $MMXY_A$ or $MMXY_B$ males, who produced lethal *MMYY* offspring when mating with *MMXY* females) and thus increased the risk of losing Y_A and/or Y_B. The fixation of X (at small N or large σ_E^2) later caused sex ratio problems after the final temperature drop



($T = -8°$) because the only genotype left (*MMXX*) then mostly produced females. As a result, populations having fixed X became extinct (white areas in fig. 3*B*) or survived under TSD with biased sex ratios (red areas). In contrast, populations that maintained Y_A and/or Y_B (large N and small σ_E^2; violet areas) could restore the initial male-heterogametic system on chromosome 1, with the masculinizing factor M fixed on chromosome 4 (i.e., *MMXX* females and *MMXY* males; the red and violet pair in fig. 2) and no sex ratio biases.

Late-Occurring Masculinizing Mutation

The end patterns in this case (fig. 3*C*) also show three domains, the boundaries of which follow those from the first set (with no masculinizing mutation; fig. 3*A*). The extinction domain was similar (white areas in both figures), but the other two domains presented different equilibrium sex-determination systems. Whenever the *mmXY* (pale green) genotype had been maintained (large N, large σ_E^2; pale green areas in fig. 3*A*), the initial male-heterogametic system on chromosome 1 was restored after fixation of M ($MMXY_{A,B}$ males and *MMXX* females; violet areas in fig. 3*C*). In contrast, in all cases in which *mmXY* (and thereby X) had been eliminated before the appearance of M (dark green in fig. 3*A*), the lethal system became fixed on chromosome 1 (with 50% offspring mortality), and sex was determined by a new male-heterogametic system on chromosome 4 (blue mMY_AY_B males and dark green mmY_AY_B females in fig. 2; blue areas in fig. 3*C*), akin to the situation currently observed in crested newts.

Discussion

As our results show, the developmental arrest syndrome found in crested newts (and related species) might be the direct consequence of the gene-environment interactions that characterize the sex-determination system of many ectothermic vertebrates. Our individual-based simulations provide a plausible scenario by which this extraordinary balanced lethal system might have evolved from ancestral sex chromosomes and thereby an example of how a maladaptive trait may evolve through natural selection.

Examples of naturally occurring balanced lethal systems are quite rare, being documented in *Drosophila tropicalis* (Dobzhansky and Pavlovsky 1955) and *Tribolium castaneum* (Dawson 1967) among insects or in *Isotoma* (James 1965) and *Oenothera* (Cleland 1972) among plants. The latter instance involves translocation heterozygosity (i.e., exchanges of segments between nonhomologous chromosomes). Although translocation-heterozygosity systems might arise by hybridization between populations having fixed alternative genotypes by drift, or following strong inbreeding in selfing populations (de Waal Malefijt and Charlesworth 1979), drift cannot account for the evolution of homozygote lethality, and the inbreeding hypothesis needs unrealistically high mutation rates and inbreeding levels to be relevant for *Triturus* (de Waal Malefijt and Charlesworth 1979).

An interesting situation, also involving sex chromosomes, occurs in the mole vole *Ellobius lutescens*. The species displays an uneven number of chromosomes ($2n = 17$), both sexes being X0 (Lyapunova and Vorontsov 1975; Fredga 1994). Hence, embryos are 25% XX, 25% 00, and 50% X0, with only X0 surviving. Whatever its evolutionary causes, this system is, however, less costly than the one under study, because embryonic mortality occurs well before the female has completed her reproductive investment. In *Triturus cristatus*, in contrast, the full investment is wasted.

Some amphibians are known to sacrifice some of their potential fertility as a part of their reproductive strategy. In the strawberry poison frog *Oophaga pumilio* (= *Dendrobates pumilio*), for instance, only 12% of the eggs laid by a female are fertilized and develop into tadpoles. The other eggs remain unfertilized and are used to feed developing larvae (Weygoldt 1980; Brust 1993). However, specific maternal oviposition strategies make sure that this investment will not benefit non-kin. In the case of *T. cristatus*, many unrelated females congregate in the same ponds to lay eggs, which makes it highly unlikely that nondeveloping embryos might preferentially benefit kin. In addition, *T. cristatus* larvae show no interest in dying embryos (P. Joly, personal communication), which are in any case protected from consumption by egg capsules. The balanced lethal system of crested newts is thus very likely to be maladaptive.

From our analyses, such a system might have evolved

Figure 3: Outcomes of simulations as a function of environmental variance for six different population sizes (N = 50, 100, 500, 1,000, 5,000, 10,000) and three different mutation scenarios: no masculinizing mutation (*A*), masculinizing mutations occurring from the start of the simulations (*B*), masculinizing mutations occurring after generation 3,000 (*C*). Color areas indicate the frequencies of different outcomes out of 100 simulations. White areas: extinctions. Dark green areas: mmY_AY_B fixed in both sexes (temperature-dependent sex determination [TSD] with lethal system). Pale green areas: mmY_AY_B males and females and $mmXY_{A,B}$ females (mixed female heterogamety with increased Y frequency and lethal system). Violet areas: *MMXX* females and $MMXY_{A,B}$ males (male heterogamety). Red areas: *MMXX* in both sexes (TSD without lethal system). Blue areas: mmY_AY_B females and mMY_AY_B males (male heterogamety on chromosome 4, with balanced lethal system on chromosome 1).

during a sex chromosome turnover induced by environmental changes. Three main mechanisms have been proposed for sex chromosome turnover, relying, respectively, on neutral processes (Scudo 1967; Bull and Charnov 1977), sex ratio selection (Hamilton 1967; Charnov 1982; Werren and Beukeboom 1998; Caubet et al. 2000; Kozielska et al. 2006; Grossen et al. 2011), and intrinsic benefits of the new sex-determining system (Bull and Charnov 1977; Orzack et al. 1980; Basolo 2001; Kraak and Pen 2002), which might stem from linkage to sex-antagonistic mutations (van Doorn and Kirkpatrick 2007). Neutral processes can be safely excluded in our case because of the strong fitness costs of this system: selective forces implied must have been strong to overcome the fitness costs of the balanced lethal system nowadays fixed. Intrinsic benefits to the new sex chromosomes, in particular through sex-antagonistic genes, are also unlikely to have driven this specific transition because the X, rather than Y, should have been fixed on the ancient pair (van Doorn and Kirkpatrick 2007).

Sex ratio selection thus appears as the most likely scenario, as our simulations also confirm. The balanced lethal system was actually fixed with high likelihood in some of our simulations sets, whenever the following two conditions were met. First, a polymorphism must preexist on the Y chromosome, with different haplotypes having fixed different deleterious mutations (such that homozygotes are lethal, while heterozygotes are viable and fully fertile). This corresponds quite precisely to the situation documented in *Poecilia reticulata* (Haskins et al. 1970 and references therein), in which several Y haplotypes that segregate in natural populations, coding for different color morphs, have been shown to be homozygous lethal. Even though the differential segment is short in guppies, it already shows some cytogenetic differentiation, with a conspicuous heterochromatic region that differs between Y haplotypes (Traut and Winking 2001). Polymorphic Y chromosomes have been documented in other species of fishes (e.g., in rainbow trout; Felip et al. 2004) and amphibians (e.g., Schmid et al. 1990; Miura 1994). Given the high drift and frequent selective sweeps expected to occur in Y chromosomes (owing to reduced effective sizes and absence of recombination), specific mechanisms might be required for the long-term maintenance of such polymorphisms. Sexual selection is a potential candidate: in natural populations of *Poecilia parae*, for instance, female preference for rare morphs mediates the coexistence of five distinct Y haplotypes, coding for distinct color morphs (Lindholm et al. 2004; Hurtado-Gonzales and Uy 2010). Alternatively, such a polymorphism might stem from secondary contacts between isolated lineages. Different populations of the female-heterogametic populations of wrinkled frog *Rana rugosa* (Miura et al. 2012) harbor different *W* haplotypes (W_N and W_K), fixed for different deleterious mutations. Experimentally produced *WW* females are viable only if heterozygous for this haplotype (W_NW_K). Note that the two haplotypes Y_A and Y_B must also present divergent inversion patterns (evolved either as a cause or as a consequence of XY recombination arrest) for recombination to be also arrested between them.

Second, environmental changes with feminizing effects must eliminate the ancestral X chromosome before a new masculinizing mutation appears (so that the population passes through a transient state of TSD). In the case of *T. cristatus*, with known thermal dependence of sex ratios (Wallace and Wallace 2000), such a shift might simply arise from a temperature drop (stemming either from climatic change or from a range expansion). The condition for the elimination of the X, however, was met only within a specific domain of population size and environmental variance (blue areas in fig. 3*C*). Population sizes and/or environmental variances that were too small resulted in extinctions due to biased and stochastic sex ratios during the TSD episode (white areas in fig. 3*C*). In contrast, too large a variance (mostly at large *N*) prevented elimination of the X, which made populations turn back to the initial *XY* system after fixation of the masculinizing mutation M (violet areas in fig. 3*C*). For the same reason, the *XY* system was maintained throughout, whenever the masculinizing mutation M appeared before X had any chance to be lost. These conditions for the balanced lethal system to evolve, however, did not require particularly small population sizes (*N* from 500 to >10,000), provided other conditions were met.

We assumed sex-reversed *XY* females to be fully fertile, which might not be the case in sex-reversed "*mM*" females (sex-reversed *T. cristatus* have been shown to have lower fitness; Wallace et al. 1997). Such an assumption, however, is conservative because low-fertility *XY* females would actually increase the probability of losing the X and thereby the probability of fixation of the balanced lethal system. Once the X was lost and the population was in a Y_AY_B TSD system (dark green in fig. 2), it had to survive a period of female-biased sex ratios, which, in some simulations, lasted for more than 2,000 generations. This certainly reduced effective population sizes but had little effect on population dynamics, given the promiscuous mating system of newts. Female biases in such cases might even boost population growth (Rankin and Kokko 2007), by increasing the absolute number of reproducing females, for a fixed carrying capacity.

This arrested-growth syndrome provides a good example of a maladaptive trait evolving through natural selection. As our simulations suggest, evolutionary outcomes as bizarre and seemingly maladaptive as the balanced lethal system of crested newts might actually be the predictable consequence of sex ratio selection (here induced by en-

vironmental changes, given gene-environment interactions on sex determination). We are not claiming, however, that our simulations provide a general scenario for the evolution of balanced lethal systems: these remain exceptional events, resulting from exceptional circumstances. The fixation of similar systems in *Oenothera*, *D. tropicalis*, or *E. lutescens* certainly occurred via alternative and species-specific pathways. In addition, a proper test of the scenario proposed here for *T. cristatus* will be difficult. The alternative scenarios (Sims et al. 1984; Wallace 1987; Sessions et al. 1988) should receive a similar formalization, in order to compare their likelihood with that of our hypothesis. Additional support might come from genomic and molecular investigations aimed at testing whether some of the genes left on chromosomal pair 1 are involved in the sex-determining cascade. The patterns of homologies between sex-linked genes in the *Triturus* phylogeny might similarly provide some insights. Sex has been assigned to a diversity of chromosome pairs in this genus (e.g., pair 2 in *T. italicus*, pair 4 in *T. alpestris*, pair 5 in *T. vulgaris* and *T. helveticus*; Mancino et al. 1977; Schmid et al. 1979), but homologies between these chromosomes are unknown. In the genus *Rana*, where homologies are better known, five different chromosome pairs have been co-opted as sex chromosomes, some of them several times independently (Miura 2007). Such a high rate of sex-chromosome turnover in amphibians is bound to blur signatures of past events, posing tough challenges to the testing of evolutionary scenarios.

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